

## **METHODS AND COMPOSITIONS FOR TREATING HYPERLIPEMIA**

### **FIELD OF THE INVENTION**

The invention relates to compositions that can ameliorate or prevent  
5 hyperlipemia and are useful as dietary supplements (e.g., health drinks) or  
medication. These compositions contain yeast cells obtainable by growth in  
electromagnetic fields with specific frequencies and field strengths.

### **BACKGROUND OF THE INVENTION**

Hyperlipemia is a state of higher than normal blood concentration of  
10 lipid components, such as cholesterols, neutral fats, phospholipids or free fatty  
acids that are in the form of water-soluble lipoproteins. Hyperlipemia is caused by  
abnormal lipoprotein metabolism. A prolonged hyperlipemic status has been linked  
to diseases of the circulatory system such as arteriosclerosis, myocardial infarction,  
angina pectoris, cerebral infarction, apoplexy, coronary diseases, cerebrovascular  
15 disorders, high blood pressure, and obesity.

Conventional therapeutic agents for the treatment of hyperlipemia  
include clofibrate, clonofibrate, phenofibrate, bezafibrate and the like, probucol and  
nicotinic acid. However, the clofibrate-type drugs are accompanied by adverse  
side effects such as formation of gallstone, muscular disorders, hepatic  
20 dysfunctions and gastrointestinal disorders. Moreover, these drugs must be  
administered in a large quantity to obtain a certain clinical effect. As a result,

severe side effects are frequently observed. There remains a need for an effective agent for treating hyperlipemia.

### **SUMMARY OF THE INVENTION**

This invention is based on the discovery that certain yeast cells can  
5 be activated by electromagnetic fields having specific frequencies and field  
strengths to produce substances useful in treating hyperlipidemia (for example,  
regulating triglyceride and/or cholesterol levels). Compositions comprising these  
activated yeast cells can therefore be used as medication or as dietary supplements,  
in the form of health drinks or dietary pills (tablets or powder). For instance, these  
10 compositions can be used to alleviate (e.g., lower) high blood lipid concentration in  
a hyperlipemic human, or to prevent or postpone the onset of hyperlipemia in a high  
risk individual (e.g., someone predisposed to hyperlipemia because of his genetic  
background or life style).

This invention embraces a composition comprising a plurality of  
15 yeast cells that have been cultured in an alternating electric field having a  
frequency in the range of about 7000 to 13000 MHz (e.g., 7500-8000, 10000-  
10500, and/or 12400-12800 MHz) and a field strength in the range of about 200 to  
450 mV/cm (e.g., 220-240, 270-290, 300-330, 310-340, 320-350, 340-370, 350-  
380, 360-390, 370-400, 390-430, and/or 420-450 mV/cm). The yeast cells are  
20 cultured for a period of time sufficient to activate said plurality of yeast cells to  
treat hyperlipemia in a subject. In one embodiment, the frequency and/or the field  
strength of the alternating electric field can be altered within the aforementioned  
ranges during said period of time. In other words, the yeast cells are exposed to a  
series of electromagnetic fields. An exemplary period of time is about 80-150  
25 hours (e.g., 100-130 hours).

Also included in this invention is a composition comprising a  
plurality of yeast cells that have been cultured under acidic conditions in an  
alternating electric field having a frequency in the range of about 12000 to 13000  
MHz (e.g., 12400-12800 MHz) and a field strength in the range of about 200 to  
30 450 mV/cm (e.g., 310-340 and/or 350-380 mV/cm). In one embodiment, the yeast

cells are exposed to a series of electromagnetic fields. An exemplary period of time is about 40-80 hours (e.g., 50-66 hours).

Yeast cells that can be included in this composition can be derived from parent strains publically available from the China General Microbiological Culture Collection Center ("CGMCC"), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China. Useful yeast species include, but are not limited to, those commonly used in food and pharmaceutical industries, such as *Saccharomyces cerevisiae* (e.g., Hansen and Hansen Var. ellipsoideus), *Saccharomyces carlsbergensis*, *Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum*, *Saccharomyces sp.*, *Schizosaccharomyces pombe*, *Rhodotorula glutinis* and *Rhodotorula aurantiaca*. For instance, the yeast cells can be of the strain *Saccharomyces cerevisiae* Hansen AS2.560 or ACCC2038, *Saccharomyces sp.* AS2.311, *Schizosaccharomyces pombe* Lindner AS2.259, *Saccharomyces sake* Yabe ACCC2045, *Saccharomyces uvarum* Beijer IFFI1036, *Saccharomyces rouxii* Boutroux AS2.371, *Saccharomyces cerevisiae* Hansen Var. ellipsoideus AS2.559, *Saccharomyces carlsbergensis* Hansen AS2.440, or *Rhodotorula glutinis* (Fresenius) Harrison AS2.704. Other useful yeast strains are illustrated in Table 1.

This invention further embraces a composition comprising a plurality of yeast cells, wherein said plurality of yeast cells have been activated to treat hyperlipemia in a subject. Included in this invention are also methods of making the above compositions.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. All publications and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting. Throughout this specification and claims, the

word “comprise,” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. A subject includes a human and veterinary subject.

5 Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 is a schematic diagram showing an exemplary apparatus for activating yeast cells using electromagnetic fields. 1: yeast culture; 2: container;  
10 3: power supply.

Fig. 2 is a schematic diagram showing an exemplary apparatus for making yeast compositions of the invention. The apparatus comprises a signal generator (such as models 83721B and 83741A manufactured by HP) and interconnected containers A, B and C.

### **DETAILED DESCRIPTION OF THE INVENTION**

15 This invention is based on the discovery that certain yeast strains can be activated by electromagnetic fields (“EMF”) having specific frequencies and field strengths to become highly efficient in producing substances that lower triglyceride and cholesterol levels in a subject. Compositions containing these  
20 activated yeast cells are useful in the treatment of hyperlipemia. Yeast compositions containing activated yeast cells can be used as medication or as dietary supplements, in the form of health drinks or dietary pills (tablets or powder).

Since the activated yeast cells contained in the yeast compositions  
25 have been cultured to endure acidic conditions (pH 2.5-4.2), these cells can survive the gastric environment and pass on to the intestines. Once in the intestines, the yeast cells are ruptured by various digestive enzymes, and the anti-hyperlipemic substances are released and readily absorbed.

#### **I. Yeast Strains Useful in the Invention**

The types of yeasts useful in this invention include, but are not limited to, yeasts of the genera *Saccharomyces*, *Schizosaccharomyces* and *Rhodotorula*.

Exemplary species within the above-listed genera include, but are not limited to, those illustrated in Table 1. Yeast strains useful for this invention can be obtained from laboratory cultures, or from publically accessible culture depositories, such as CGMCC and the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209. Non-limiting examples of useful strains (with accession numbers of CGMCC) are *Saccharomyces cerevisiae* Hansen AS2.560 and ACCC2038, *Saccharomyces sp.* AS2.311, *Schizosaccharomyces pombe* Lindner AS2.259, *Saccharomyces sake* Yabe ACCC2045, *Saccharomyces uvarum* Beijer IFFI1036, *Saccharomyces rouxii* Boutroux AS2.371, *Saccharomyces cerevisiae* Hansen Var. ellipsoideus AS2.559, *Saccharomyces carlsbergensis* Hansen AS2.440, and *Rhodotorula glutinis* (Fresenius) Harrison AS2.704. Other useful yeast strains are illustrated in Table 1.

The preparation of the yeast compositions of this invention is not limited to starting with a pure strain of yeast. A yeast composition of the invention may be produced by culturing a mixture of yeast cells of different species or strains. The ability of any activated species or strain of yeasts to treat hyperlipemia can be readily tested by methods known in the art. See, for instance, Examples 1 and 2.

Table 1 Exemplary Yeast Strains

<i>Saccharomyces cerevisiae</i> Hansen					
	ACCC2034	ACCC2035	ACCC2036	ACCC2037	ACCC2038
	ACCC2039	ACCC2040	ACCC2041	ACCC2042	AS2.1
5	AS2.4	AS2.11	AS2.14	AS2.16	AS2.56
	AS2.69	AS2.70	AS2.93	AS2.98	AS2.101
	AS2.109	AS2.110	AS2.112	AS2.139	AS2.173
	AS2.174	AS2.182	AS2.196	AS2.242	AS2.336
	AS2.346	AS2.369	AS2.374	AS2.375	AS2.379
10	AS2.380	AS2.382	AS2.390	AS2.393	AS2.395
	AS2.396	AS2.397	AS2.398	AS2.399	AS2.400
	AS2.406	AS2.408	AS2.409	AS2.413	AS2.414
	AS2.415	AS2.416	AS2.422	AS2.423	AS2.430
	AS2.431	AS2.432	AS2.451	AS2.452	AS2.453
15	AS2.458	AS2.460	AS2.463	AS2.467	AS2.486
	AS2.501	AS2.502	AS2.503	AS2.504	AS2.516
	AS2.535	AS2.536	AS2.558	AS2.560	AS2.561
	AS2.562	AS2.576	AS2.593	AS2.594	AS2.614
	AS2.620	AS2.628	AS2.631	AS2.666	AS2.982
20	AS2.1190	AS2.1364	AS2.1396	IFFI1001	IFFI1002
	IFFI1005	IFFI1006	IFFI1008	IFFI1009	IFFI1010
	IFFI1012	IFFI1021	IFFI1027	IFFI1037	IFFI1042
	IFFI1043	IFFI1045	IFFI1048	IFFI1049	IFFI1050
	IFFI1052	IFFI1059	IFFI1060	IFFI1062	IFFI1063
25	IFFI1202	IFFI1203	IFFI1206	IFFI1209	IFFI1210
	IFFI1211	IFFI1212	IFFI1213	IFFI1214	IFFI1215
	IFFI1220	IFFI1221	IFFI1224	IFFI1247	IFFI1248
	IFFI1251	IFFI1270	IFFI1277	IFFI1287	IFFI1289
	IFFI1290	IFFI1291	IFFI1292	IFFI1293	IFFI1297
30	IFFI1300	IFFI1301	IFFI1302	IFFI1307	IFFI1308
	IFFI1309	IFFI1310	IFFI1311	IFFI1331	IFFI1335

	IFFI1336	IFFI1337	IFFI1338	IFFI1339	IFFI1340
	IFFI1345	IFFI1348	IFFI1396	IFFI1397	IFFI1399
	IFFI1411	IFFI1413	IFFI1441	IFFI1443	
	<i>Saccharomyces cerevisiae</i> Hansen Var. ellipsoideus (Hansen) Dekker				
5	ACCC2043	AS2.2	AS2.3	AS2.8	AS2.53
	AS2.163	AS2.168	AS2.483	AS2.541	AS2.559
	AS2.606	AS2.607	AS2.611	AS2.612	
	<i>Saccharomyces chevalieri</i> Guilliermond				
	AS2.131	AS2.213			
10	<i>Saccharomyces delbrueckii</i>				
	AS2.285				
	<i>Saccharomyces delbrueckii</i> Lindner ver. mongolicus (Saito) Lodder et van Rij				
	AS2.209	AS2.1157			
	<i>Saccharomyces exiguus</i> Hansen				
15	AS2.349	AS2.1158			
	<i>Saccharomyces fermentati</i> (Saito) Lodder et van Rij				
	AS2.286	AS2.343			
	<i>Saccharomyces logos</i> van laer et Denamur ex Jorgensen				
	AS2.156	AS2.327	AS2.335		
20	<i>Saccharomyces mellis</i> (Fabian et Quinet) Lodder et kreger van Rij				
	AS2.195				
	<i>Saccharomyces mellis</i> Microellipsoides Osterwalder				
	AS2.699				
	<i>Saccharomyces oviformis</i> Osteralder				
25	AS2.100				
	<i>Saccharomyces rosei</i> (Guilliermond) Lodder et Kreger van Rij				
	AS2.287				
	<i>Saccharomyces rouxii</i> Boutroux				
	AS2.178	AS2.180	AS2.370	AS2.371	
30	<i>Saccharomyces sake</i> Yabe				

	ACCC2045				
	<i>Candida arborea</i>				
	AS2.566				
	<i>Candida lambica</i> (Lindner et Genoud) van. Uden et Buckley				
5	AS2.1182				
	<i>Candida krusei</i> (Castellani) Berkhout				
	AS2.1045				
	<i>Candida lipolytica</i> (Harrison) Diddens et Lodder				
	AS2.1207	AS2.1216	AS2.1220	AS2.1379	AS2.1398
10	AS2.1399	AS2.1400			
	<i>Candida parapsilosis</i> (Ashford) Langeron et Talice Var. intermedia Van Rij et Verona				
	AS2.491				
	<i>Candida parapsilosis</i> (Ashford) Langeron et Talice				
15	AS2.590				
	<i>Candida pulcherrima</i> (Lindner) Windisch				
	AS2.492				
	<i>Candida rugosa</i> (Anderson) Diddens et Lodder				
	AS2.511	AS2.1367	AS2.1369	AS2.1372	AS2.1373
20	AS2.1377	AS2.1378	AS2.1384		
	<i>Candida tropicalis</i> (Castellani) Berkhout				
	ACCC2004	ACCC2005	ACCC2006	AS2.164	AS2.402
	AS2.564	AS2.565	AS2.567	AS2.568	AS2.617
	AS2.637	AS2.1387	AS2.1397		
25	<i>Candida utilis</i> Henneberg Lodder et Kreger Van Rij				
	AS2.120	AS2.281	AS2.1180		
	<i>Crebrothecium ashbyii</i> (Guilliermond) Routein ( <i>Eremothecium ashbyii</i> Guilliermond)				
	AS2.481	AS2.482	AS2.1197		
30	<i>Geotrichum candidum</i> Link				



	ACCC2016	AS2.361	AS2.498	AS2.616	AS2.1035
	AS2.1062	AS2.1080	AS2.1132	AS2.1175	AS2.1183
	<i>Hansenula anomala</i> (Hansen)H et P sydow				
5	ACCC2018	AS2.294	AS2.295	AS2.296	AS2.297
	AS2.298	AS2.299	AS2.300	AS2.302	AS2.338
	AS2.339	AS2.340	AS2.341	AS2.470	AS2.592
	AS2.641	AS2.642	AS2.782	AS2.635	AS2.794
	<i>Hansenula arabitolgens</i> Fang				
	AS2.887				
10	<i>Hansenula jadinii</i> (A. et R Sartory Weill et Meyer) Wickerham				
	ACCC2019				
	<i>Hansenula saturnus</i> (Klocker) H et P sydow				
	ACCC2020				
	<i>Hansenula schneeggii</i> (Weber ) Dekker				
15	AS2.304				
	<i>Hansenula subpelliculosa</i> Bedford				
	AS2.740	AS2.760	AS2.761	AS2.770	AS2.783
	AS2.790	AS2.798	AS2.866		
	<i>Kloeckera apiculata</i> (Reess emend. Klocker) Janke				
20	ACCC2022	ACCC2023	AS2.197	AS2.496	AS2.714
	ACCC2021	AS2.711			
	<i>Lipomycess starkeyi</i> Lodder et van Rij				
	AS2.1390	ACCC2024			
	<i>Pichia farinosa</i> (Lindner) Hansen				
25	ACCC2025	ACCC2026	AS2.86	AS2.87	AS2.705
	AS2.803				
	<i>Pichia membranaefaciens</i> Hansen				
	ACCC2027	AS2.89	AS2.661	AS2.1039	
	<i>Rhodospiridium toruloides</i> Banno				
30	ACCC2028				

	<i>Rhodotorula glutinis</i> (Fresenius) Harrison				
	AS2.2029	AS2.280	ACCC2030	AS2.102	AS2.107
	AS2.278	AS2.499	AS2.694	AS2.703	AS2.704
	AS2.1146				
5	<i>Rhodotorula minuta</i> (Saito) Harrison				
	AS2.277				
	<i>Rhodotorula rubra</i> (Demme) Lodder				
	AS2.21	AS2.22	AS2.103	AS2.105	AS2.108
	AS2.140	AS2.166	AS2.167	AS2.272	AS2.279
10	AS2.282	ACCC2031			
	<i>Rhodotorula aurantiaca</i> (Saito) Lodder				
	AS2.102	AS2.107	AS2.278	AS2.499	AS2.694
	AS2.703	AS2.1146			
	<i>Saccharomyces carlsbergensis</i> Hansen				
15	AS2.113	ACCC2032	ACCC2033	AS2.312	AS2.116
	AS2.118	AS2.121	AS2.132	AS2.162	AS2.189
	AS2.200	AS2.216	AS2.265	AS2.377	AS2.417
	AS2.420	AS2.440	AS2.441	AS2.443	AS2.444
	AS2.459	AS2.595	AS2.605	AS2.638	AS2.742
20	AS2.745	AS2.748	AS2.1042		
	<i>Saccharomyces uvarum</i> Beijer				
	IFFI1023	IFFI1032	IFFI1036	IFFI1044	IFFI1072
	IFFI1205	IFFI1207			
	<i>Saccharomyces willianus</i> Saccardo				
25	AS2.5	AS2.7	AS2.119	AS2.152	AS2.293
	AS2.381	AS2.392	AS2.434	AS2.614	AS2.1189
	<i>Saccharomyces sp.</i>				
	AS2.311				
	<i>Saccharomycodes ludwigii</i> Hansen				
30	ACCC2044	AS2.243	AS2.508		

	<i>Saccharomyces sinenses</i> Yue				
	AS2.1395				
	<i>Schizosaccharomyces octosporus</i> Beijerinck				
	ACCC2046 AS2.1148				
5	<i>Schizosaccharomyces pombe</i> Lindner				
	ACCC2047	ACCC2048	AS2.214	AS2.248	AS2.249
	AS2.255	AS2.257	AS2.259	AS2.260	AS2.274
	AS2.994	AS2.1043	AS2.1149	AS2.1178	IFFI1056
	<i>Sporobolomyces roseus</i> Kluyver et van Niel				
10	ACCC2049	ACCC2050	AS2.19	AS2.962	AS2.1036
	ACCC2051	AS2.261	AS2.262		
	<i>Torulopsis candida</i> (Saito) Lodder				
	AS2.270	ACCC2052			
	<i>Torulopsis famta</i> (Harrison) Lodder et van Rij				
15	ACCC2053	AS2.685			
	<i>Torulopsis globosa</i> (Olson et Hammer) Lodder et van Rij				
	ACCC2054	AS2.202			
	<i>Torulopsis inconspicua</i> Lodder et Kreger van Rij				
	AS2.75				
20	<i>Trichosporon behrendii</i> Lodder et Kreger van Rij				
	ACCC2056	AS2.1193			
	<i>Trichosporon capitatum</i> Diddens et Lodder				
	ACCC2056	AS2.1385			
	<i>Trichosporon cutaneum</i> (de Beurm et al.) Ota				
25	ACCC2057	AS2.25	AS2.570	AS2.571	AS2.1374
	<i>Wickerhamia fluorescens</i> (Soneda) Soneda				
	ACCC2058	AS2.1388			

## II. Application of Electromagnetic Fields

An electromagnetic field useful in this invention can be generated and applied by various means well known in the art. For instance, the EMF can be generated by applying an alternating electric field or an oscillating magnetic field.

5 Alternating electric fields can be applied to cell cultures through electrodes in direct contact with the culture medium, or through electromagnetic induction. See, e.g., Fig. 1. Relatively high electric fields in the medium can be generated using a method in which the electrodes are in contact with the medium. Care must be taken to prevent electrolysis at the electrodes from introducing  
10 undesired ions into the culture and to prevent contact resistance, bubbles, or other features of electrolysis from dropping the field level below that intended. Electrodes should be matched to their environment, for example, using Ag-AgCl electrodes in solutions rich in chloride ions, and run at as low a voltage as possible. For general review, see Goodman et al., *Effects of EMF on Molecules and Cells*,  
15 International Review of Cytology, A Survey of Cell Biology, Vol. 158, Academic Press, 1995.

The EMFs useful in this invention can also be generated by applying an oscillating magnetic field. An oscillating magnetic field can be generated by oscillating electric currents going through Helmholtz coils. Such a  
20 magnetic field in turn induces an electric field.

The frequencies of EMFs useful in this invention range from about 7000-13000 MHz (e.g., 7500-8000, 10000-10500, and/or 12400-12800 MHz). Exemplary frequencies are 7858, 7873, 10072, 12623, and 12642 MHz. The field strength of the electric field useful in this invention ranges from about 200 to 450  
25 mV/cm (e.g., 220-240, 270-290, 300-330, 310-340, 320-350, 340-370, 350-380, 360-390, 370-400, 390-430, and/or 420-450 mV/cm). Exemplary field strengths are 228, 276, 284, 305, 317, 320, 332, 346, 363, 374, 384, and 407 mV/cm.

When a series of EMFs are applied to a yeast culture, the yeast culture can remain in the same container while the same set of EMF generator and  
30 emitters is used to change the frequency and/or field strength. The EMFs in the series can each have a different frequency or a different field strength; or a different frequency and a different field strength. Such frequencies and field

strengths are preferably within the above-described ranges. Although any practical number of EMFs can be used in a series, it may be preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or more EMFs in a series. In one embodiment, the yeast culture is exposed to a series of EMFs,  
5 wherein the frequency of the electric field is alternated in the range of about 7500-8000, 10000-10500, and 12400-12800 MHz.

Although the yeast cells can be activated after even a few hours of culturing in the presence of an EMF, it may be preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EMF(s) for a total of  
10 80-150 hours (e.g., 100-130 hours).

Fig. 1 illustrates an exemplary apparatus for generating alternating electric fields. An electric field of a desired frequency and intensity is generated by an AC source (3) capable of generating an alternating electric field, preferably in a sinusoidal wave form, in the frequency range of 10 to 20,000 MHz. Signal  
15 generators capable of generating signals with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output. The activation container (2) can be made from non-conductive material, for example, plastics, glass steel, ceramic, and combinations thereof. The wire connecting the activation container (2) and the signal generator (3) is preferably a  
20 high frequency coaxial cable with a transmission frequency of at least 30 GHz.

The alternating electric field can be applied to the culture by a variety of means, including placing the yeast culture (1) in close proximity to the signal emitters such as a metal wire or tube capable of transmitting EMFs. The metal wire or tube can be made of red copper, and be placed inside the container  
25 (2), reaching as deep as 3-30 cm. For example, if the fluid in the container (2) has a depth of 15-20 cm, 20-30 cm, 30-50 cm, 50-70 cm, 70-100 cm, 100-150 cm or 150-200 cm, the metal wire can be 3-5 cm, 5-7 cm, 7-10 cm, 10-15 cm; 15-20 cm, 20-30 cm and 25-30 cm from the bottom of the container (2), respectively. The number of electrode wires used depends on the volume of the culture as well as the  
30 diameter of the wires. The number of metal wires/tubes used can be from 1 to 10 (e.g., 2 to 3). It is recommended, though not mandated, that for a culture having a volume up to 10 L, metal wires/tubes having a diameter of 0.5 to 2.0 mm be used.

For a culture having a volume between 10 L and 100 L, metal wires/tubes having a diameter of 3.0 to 5.0 mm can be used. For a culture having a volume in the range of 100-1000 L, metal wires/tubes having a diameter of 6.0 to 15.0 mm can be used. For a culture having a volume greater than 1000 L, metal wires/tubes having a  
5 diameter of 20.0 to 25.0 mm can be used.

In one embodiment, the electric field is applied by electrodes submerged in the culture (1). In this embodiment, one of the electrodes can be a metal plate placed on the bottom of the container (2), and the other electrode can comprise a plurality of electrode wires evenly distributed in the culture (1) so as to  
10 achieve even distribution of the electric field energy. The number of electrode wires used depends on the volume of the culture as well as the diameter of the wires.

### **III. Culture Media**

Culture media useful in this invention contain sources of nutrients  
15 assimilable by yeast cells. Complex carbon-containing substances in a suitable form, such as carbohydrates (e.g., sucrose, glucose, fructose, dextrose, maltose, xylose, cellulose, starches, etc.) and coal, can be the carbon sources for yeast cells. The exact quantity of the carbon sources utilized in the medium can be adjusted in accordance with the other ingredients of the medium. In general, the amount of  
20 carbohydrates varies between about 0.1% and 10% by weight of the medium and preferably between about 0.1% and 5% (e.g., about 2%). These carbon sources can be used individually or in combination. Amino acid-containing substances in suitable form (e.g., beef extract and peptone) can also be added individually or in combination. In general, the amount of amino acid containing substances varies  
25 between about 0.1% and 0.5% by weight of the medium and preferably between about 0.1% and 0.3% (e.g., about 0.25%). Among the inorganic salts which can be added to the culture medium are the customary salts capable of yielding sodium, potassium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $(\text{NH}_4)_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{CaCO}_3$ ,  
30  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

### **IV. Electromagnetic Activation of Yeast Cells**

To activate or enhance the ability of yeast cells to produce substances beneficial for the treatment of hyperlipemia (e.g., lowering of triglyceride and/or cholesterol levels), these cells can be activated by being cultured in an appropriate medium under sterile conditions at 20°C-38°C, preferably at 28-32°C (e.g., 30°C) for a sufficient amount of time, e.g., 100-200 hours (e.g., 147-171 hours), in an alternating electric field or a series of alternating electric fields as described above.

An exemplary culture medium is made by mixing 950 ml of distilled water with 20 g of sucrose, 20 µg of vitamin B<sub>12</sub>, 40 µg of vitamin B<sub>6</sub>, 100 µg of vitamin E, 50 ml of fetal bovine serum, 0.20 g of KH<sub>2</sub>PO<sub>4</sub>, 0.25 g of MgSO<sub>4</sub>•7H<sub>2</sub>O, 0.3 g of NaCl, 0.2 g of CaSO<sub>4</sub>•2H<sub>2</sub>O, 4.0 g of CaCO<sub>3</sub>•5H<sub>2</sub>O, and 2.5 g of peptone.

An exemplary set-up of the culturing process is depicted in Fig. 1. Untreated yeast cells are added to a culture medium at  $1 \times 10^8$  cells per 1000 ml of the culture medium. The yeast cells may be *Saccharomyces cerevisiae* Hansen AS2.560, or may be selected from any of the strains listed in Table 1. An exemplary activation process of the yeast cells involves the following sequence: the yeast cells are grown in the culture medium for 26-30 hours (e.g., 28 hours) at 28-32°C and then exposed to (1) an alternating electric field having a frequency of 7858 MHz and a field strength in the range of 270-290 mV/cm (e.g., 276 mV/cm) for 14-18 hours (e.g., 16 hours); (2) then to an alternating electric field having a frequency of 7873 MHz and a field strength in the range of 300-330 mV/cm (e.g., 305 mV/cm) for 30-34 hours (e.g., 32 hours); (3) then to an alternating electric field having a frequency of 10072 MHz and a field strength in the range of 300-330 mV/cm (e.g., 317 mV/cm) for 37-41 hours (e.g., 39 hours); (4) then to an alternating electric field having a frequency of 12623 MHz and a field strength in the range of 340-370 mV/cm (e.g., 346 mV/cm) for 27-31 hours (e.g., 29 hours); and (5) finally to an alternating electric field having a frequency of 12642 MHz and a field strength in the range of 270-290 mV/cm (e.g., 284 mV/cm) for 13-17 hours (e.g., 15 hours). The activated yeast cells are then recovered from the culture medium by various methods known in the art, dried (e.g., by lyophilization)

and stored at about 4°C in powder form. The resultant yeast powder preferably contains no less than  $10^{10}$  cells/g activated yeast.

Subsequently, the activated yeast cells can be evaluated for their ability to treat hyperlipemia using standard methods known in the art, such as those  
5 described in Section VII.

**V. Acclimatization of Yeast Cells To the Gastric Environment**

Because the activated yeast cells of this invention must pass through the stomach before reaching the small intestine, where the effective components are released from these yeast cells, it is preferred that these yeasts be cultured  
10 under acidic conditions so as to acclimatize the cells to the gastric juice. This acclimatization process results in better viability of the yeast cells in the acidic gastric environment.

To achieve this, the yeast powder containing activated yeast cells can be mixed with a highly acidic acclimatizing culture medium at 10 g  
15 (containing more than  $10^{10}$  activated cells per gram) per 1000 ml. The yeast mixture can then be cultured first in the presence of an alternating electric field having a frequency of 12623 MHz and a field strength in the range of 350-380 mV/cm (e.g., 363 mV/cm) at about 28 to 32°C for 34-42 hours (e.g., 38 hours). The resultant yeast cells can then be further incubated in the presence of an  
20 alternating electric field having a frequency of 12642 MHz and a field strength in the range of 310-340 mV/cm (e.g., 320 mV/cm) at about 28 to 32°C for 16-24 hours (e.g., 20 hours). The resulting acclimatized yeast cells are then recovered from the culture medium by various methods known in the art and are dried and stored either in powder form ( $\geq 10^{10}$  cells/g) at room temperature or in vacuum at 0-  
25 4°C.

An exemplary acclimatizing culture medium is made by mixing 700 ml fresh pig gastric juice and 300 ml wild Chinese hawthorn extract. The pH of acclimatizing culture medium is adjusted to 2.5 with 0.1 M hydrochloric acid (HCl) and 0.2 M potassium hydrogen phthalate ( $C_6H_4(COOK)COOH$ ). The fresh  
30 pig gastric juice is prepared as follows. At about 4 months of age, newborn Holland white pigs are sacrificed, and the entire contents of their stomachs are retrieved and mixed with 2000 ml of water under sterile conditions. The mixture is



then allowed to stand for 6 hours at 4°C under sterile conditions to precipitate food debris. The supernatant is collected for use in the acclimatizing culture medium.

To prepare the wild Chinese hawthorn extract, 500 g of fresh wild Chinese hawthorn is dried under sterile conditions to reduce water content ( $\leq 8\%$ ). The  
5 dried fruit is then ground ( $\geq 20$  mesh) and added to 1500 ml of sterile water. The hawthorn slurry is allowed to stand for 6 hours at 4°C under sterile conditions. The hawthorn supernatant is collected to be used in the acclimatizing culture medium.

#### VI. Manufacture of Yeast Compositions

10 To prepare the yeast compositions of the invention, an apparatus depicted in Fig. 2 or an equivalent thereof can be used. This apparatus includes three containers, a first container (A), a second container (B), and a third container (C), each equipped with a pair of electrodes (4). One of the electrodes is a metal plate placed on the bottom of the containers, and the other electrode comprises a  
15 plurality of electrode wires evenly distributed in the space within the container to achieve even distribution of the electric field energy. All three pairs of electrodes are connected to a common signal generator.

The culture medium used for this purpose is a mixed fruit extract solution containing the following ingredients per 1000 L: 300 L of wild Chinese  
20 hawthorn extract, 300 L of jujube extract, 300 L of *Schisandra chinensis* (Turez) Baill seeds extract, and 100 L of soy bean extract. To prepare hawthorn, jujube and *Schisandra chinensis* (Turez) Baill seeds extracts, the fresh fruits are washed and dried under sterile conditions to reduce the water content to no higher than 8%. One hundred kilograms of the dried fruits are then ground ( $\geq 20$  mesh) and added to  
25 400 L of sterile water. The mixtures are stirred under sterile conditions at room temperature for twelve hours, and then centrifuged at 1000 rpm to remove insoluble residues. To make the soy bean extract, fresh soy beans are washed and dried under sterile conditions to reduce the water content to no higher than 8%. Thirty kilograms of dried soy beans are then ground into particles of no smaller  
30 than 20 mesh, and added to 130 L of sterile water. The mixture is stirred under sterile conditions at room temperature for twelve hours and centrifuged at 1000 rpm to remove insoluble residues. Once the mixed fruit extract solution is

prepared, it is autoclaved at 121°C for 30 minutes and cooled to below 40°C before use.

One thousand grams of the activated yeast powder prepared as described above (Section V, *supra*) is added to 1000 L of the mixed fruit extract solution, and the yeast solution is transferred to the first container (A) shown in Fig. 2. The yeast cells are then cultured in the presence of an alternating electric field having a frequency of 12623 MHz and a field strength of about 370-400 mV/cm (e.g., 384 mV/cm) at 28-32°C under sterile conditions for 26 hours. The yeast cells are further incubated in an alternating electric field having a frequency of 12642 MHz and a field strength of 320-350 mV/cm (e.g., 332 mV/cm). The culturing continues for another 12 hours.

The yeast culture is then transferred from the first container (A) to the second container (B) (if need be, a new batch of yeast culture can be started in the now available the first container (A)), and subjected to an alternating electric field having a frequency of 12623 MHz and a field strength of 390-430 mV/cm (e.g., 407 mV/cm) for 24 hours. Subsequently the frequency and field strength of the electric field are changed to 12642 MHz and 360-390 mV/cm (e.g., 374 mV/cm), respectively. The culturing process continues for another 12 hours.

The yeast culture is then transferred from the second container (B) to the third container (C), and subjected to an alternating electric field having a frequency of 12623 MHz and a field strength of 270-290 mV/cm (e.g., 276 mV/cm) for 24 hours. Subsequently the frequency and field strength of the electric field are changed to 12642 MHz and 220-240 mV/cm (e.g., 228 mV/cm), respectively. The culturing continues for another 12 hours.

The yeast culture from the third container (C) can then be packaged into vacuum sealed bottles for use as medicament or dietary supplement, e.g., in the form of health drinks, pills, or powder, etc. If desired, the final yeast culture can also be dried within 24 hours and stored in powder form. The dietary supplement can be taken three to four times daily at 30~60 ml per dose for a three-month period, preferably 10-30 minutes before meals and at bedtime.

In some embodiments, the compositions of the invention can also be administered intravenously or peritoneally in the form of a sterile injectable

preparation. Such a sterile preparation can be prepared as follows. A sterilized health drink composition is first treated under ultrasound ( $\geq 18,000$  Hz) for 10 minutes and then centrifuged for another 10 minutes. The resulting supernatant is adjusted to pH 7.2-7.4 using 1 M NaOH and subsequently filtered through a  
5 membrane (0.22  $\mu\text{m}$  for intravenous injection and 0.45  $\mu\text{m}$  for peritoneal injection) under sterile conditions. The resulting sterile preparation is submerged in a 35-38  $^{\circ}\text{C}$  water bath for 30 minutes before use. In other embodiments, the compositions of the invention may also be formulated with pharmaceutically acceptable carriers to be orally administered in any orally acceptable dosage form including, but not  
10 limited to, capsules, tablets, suspensions or solutions.

The yeast compositions of the present invention are derived from yeasts used in food and pharmaceutical industries. The yeast compositions are thus devoid of side effects associated with many pharmaceutical compounds.

## **VII. Examples**

15 In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

The activated yeast compositions used in the following examples were prepared as described above, using *Saccharomyces cerevisiae* Hansen  
20 AS2.560 cells, cultured in the presence of an alternating electric field having the electric field frequency and field strength exemplified in the parentheses following the recommended ranges listed in Section IV, *supra*. Control (i.e., untreated) yeast compositions were those prepared in the same manner as described in Section VI, *supra*, except that the yeast cells were cultured in the absence of EMFs. All  
25 compositions of interest were administered to the animals by intragastric feeding, unless otherwise specified.

### **Example 1: Effects of the Treatment on Cholesterol Levels in Rabbits**

To test the ability of the activated yeast compositions to control hyperlipemia, thirty healthy domesticated rabbits (*Oryctolagus curiculus*, 3.8-4.2  
30 kg, half of them male and half of them female, 18-24 months old) were given regular rabbit feed for four weeks and then were randomly divided into three groups, Group A, B and C. A mixture of 0.2 mg/kg cholesterol + 2 ml lard was

administered to each rabbit once daily and a composition of interest at a dosage of 1.5 ml/kg was administered twice a day for eight consecutive weeks. Rabbits in Groups A, B and C were given the activated yeast composition, the control yeast composition and saline, respectively, in addition to cholesterol and lard. All rabbits were otherwise maintained the same way.

Prior to the administration of the mixture and the composition of interest, as well as at 2, 4, 6 and 8 weeks after the administration, blood samples were taken from the marginal vein of an ear of each rabbit and centrifuged at 3000 rpm to recover sera. The amount of serum cholesterol was measured by the method described in Table 2 below.

Table 2. A Method for Determining Cholesterol Concentration.

Steps	Contents in Each Test Tube	
	Standard Tube (ml)	Sample Tube (ml)
Mixed Serum,	-	0.2
Cholesterol Standard Solution, (2 mg/ml)	0.2	-
Glacial Acetic Acid, (ml)	0.5	0.5
0.9 % NaCl,	0.2	-
and Acetic Anhydride	4.8	5.0
Mixed Vigorously for 5 Minutes and Centrifuged for 5 Minutes		
To the resulting Top Layer,	4.0	4.0
Added conc. H <sub>2</sub> SO <sub>4</sub> (ml)	0.2	0.2
Stirred in a 25°C water bath for 15 minutes. Readings of the sample and standard tubes were taken with a spectrophotometer at 630 nm (calibrated against blank control with distilled water).		

A standard curve of cholesterol was established and the concentration of cholesterol in the samples was determined according to the standard curve. The results were shown in Table 3.

Table 3. Effects on Cholesterol Concentration

Group	Cholesterol (mg/100ml)				
	Day 1	2 weeks	4 weeks	6 weeks	8 weeks
A	83.87± 15.42	242.43± 23.64	156.55± 21.34	98.73± 11.78	87.37± 8.79
B	79.53± 14.22	383.65± 29.54	643.56± 65.45	703.67± 64.78	778.76± 86.87
C	77.46± 11.31	377.67± 30.26	612.87± 89.54	712.32± 98.35	823.78± 102.54

The above results show that the activated yeast composition was more effective in lowering the cholesterol levels than the control yeast composition.

**Example 2: Effects of the Treatment on Cholesterol and Triglyceride Levels in Rats**

To test the ability of the activated yeast compositions to control hyperlipemia, forty healthy Wistar rats (120-140 g, half of them male and half of them female, 2-4 months old) were randomly divided into four groups, Groups A, B, C and D. Groups A, B and C rats were given high lipid content rat feed and Groups D rats were given regular rat feed for three weeks. A composition of interest at a dosage of 1.5 ml/100 g was then administered to each rat in Groups A, B and C once daily in conjunction with the high lipid content rat feed for another three consecutive weeks. Rats in Group A, B and C were given the activated yeast composition, the control yeast composition and saline, respectively, in addition to the high lipid content rat feed. Rats in Group D were given 1.5 ml/100 g saline and regular rat feed instead. The high lipid content rat feed was prepared by mixing 1% cholesterol, 0.5% bile salt, 0.2% methylthiouracil, 2% lard, 5% soybean, 1% egg, 2% ground dry yolk, 1% fish meal, and 87.3% regular rat feed with water to form a paste.

Twenty-four hours after the last administration of the composition, the rats were given no feed but water for another twelve hours. Blood samples were then taken from the carotid and jugular vein of the rat and centrifuged at 3000 rpm to recover sera. The amount of serum cholesterol was measured by the same

method as described in Example 1. The amount of serum triglycerides was measured by the method described in Table 4 below.

Table 4. A Method for Determining Triglyceride Concentration.

	Steps	Contents in Each Test Tube		
		Blank Control Tube (ml)	Standard Tube (ml)	Sample Tube (ml)
5	Mixed Serum,	-	-	0.2
	Triglyceride Standard Solution, (1.0 mg/ml)	-	0.2	-
10	Distilled Water (ml)	0.2	0.2	-
	with the Extracting Solvent (1:1 v/v n-heptane/i-PrOH)	2.2	2.0	2.2
	Shook vigorously so that triglycerides dissolved in the extracting solvent			
15	Added H <sub>2</sub> SO <sub>4</sub> (0.04 M)	0.5	0.5	0.5
	Shook vigorously for a few seconds and allowed the mixture to separate into two layers			
20	To the Resulting Top Layer	0.4	0.4	0.4
	Added 5% KOH	2.0	2.0	2.0
	Stirred in a 65°C water bath for 5 minutes to allow saponification			
	Added NaIO <sub>4</sub> (0.2 M)	1.0	1.0	1.0
25	The mixture was stirred			
	Added Acetylicetone (0.5 M)	1.0	1.0	1.0
30	Stirred in a 65°C water bath for 10 minutes and cooled to room temperature. Readings of the sample and standard tubes were taken with a spectrophotometer at 420 nm (calibrated with the blank control tube).			

A standard curve of triglyceride was established and the concentration of triglyceride in the samples was determined according to the standard curve. The results were shown in Table 5.

Table 5. Effects on Triglyceride and Cholesterol Concentrations

5	Group	Cholesterol (mg/100ml)	Triglyceride (mg/100ml)
	A	126.4±43.7	87.4±12.6
	B	734.6±214.7	223.6±127.8
	C	723.8±221.6	213.4±146.7
	D	98.8±36.7	59.7±22.6

10                      The above results show that the activated yeast composition was more effective in lowering the triglyceride and cholesterol levels than the control yeast composition.

15                      While a number of embodiments of this invention have been set forth, it is apparent that the basic constructions may be altered to provide other embodiments which utilize the compositions and methods of this invention.